Chemical Composition and Insecticidal Activity of Essential Oil from *Syzygium aromaticum* (Myrtaceae) against the Sawtoothed Grain Beetle *Oryzaephilus surinamensis* (Coleoptera: Silvanidae)[1]

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J. Entomol. Sci. 60(2): 276–286 (April 2025)
DOI: 10.18474/JES24-33

Abstract Utilizing essential oils extracted from specific herbal plants offers an intriguing alternative to synthetic insecticides, which are known for their harmful effects on both consumers and the environment, in preventing the devastation caused by the sawtoothed grain beetle, *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae). This study aimed to explore the chemical composition and insecticidal activities of essential oil derived from *Syzygium aromaticum* (L.) Merrill & L.M. Perry against adults of *O. surinamensis*. The research involved assessing the chemical compound of the essential oil from the flower buds of *S. aromaticum*, evaluating its fumigant toxicity and its repellent effect on *O. surinamensis* adults through vapor-phase testing conducted in laboratory conditions at 30°C ± 5°C and relative humidity of 70% ± 5%. The experimental design used a completely randomized design with four replications and six concentrations (0, 10, 20, 30, 40, and 50 μl/l of air). A total of nine chemical constituents was identified, with eugenol (90.15%) emerging as the primary compound in *S. aromaticum* essential oil. The fumigation toxicity (50% lethal concentration) assessments on adult *O. surinamensis* at 24, 48, and 72 h revealed values of 9.70, 6.58, and 4.37 μl/l of air, respectively. Over the 24- to 120-h test period, the application of *S. aromaticum* essential oil at 50 μl/l of air resulted in the highest adult mortality among *O. surinamensis*. Notably, at a concentration of 40 μl/l of air, the essential oil of *S. aromaticum* demonstrated a fumigation efficiency of 90–100% at both 24 and 72 h, showing no significant difference compared with a concentration 50 μl/l of air. These findings highlight the potential of *S. aromaticum* essential oil as an effective insecticide for controlling *O. surinamensis* populations in agricultural storage.

Key Words spicy plant, essential oil, stored-product insect, toxicity, chemical composition

In agricultural environments, insect pests pose a significant threat to stored grains, resulting in considerable losses in both quantity and quality of product.

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Among these pests, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), commonly known as the sawtoothed grain beetle, infests stored grains, causing damage to their viability and weight, while also introducing foreign matter that poses health risks to consumers (Perera and Karunaratne 2015). Effective pest management strategies are crucial to mitigate the destructive impact of insect infestations in storage facilities. With the phasing out of synthetic fumigants such as methyl bromide because of environmental concerns and the rise of resistance in pests to chemical insecticides, the necessity for sustainable alternatives has become increasingly evident (Rajashekar et al. 2016). Natural botanical extracts, especially essential oils, have emerged as promising options for controlling stored product pests (Gitahi et al. 2021). Essential oils possess diverse bioactivities, including insecticidal, repellent, and oviposition deterrent properties, making them valuable tools in integrated pest management (IPM) programs (Isman 2020, Shaw and Catteruccia 2019).

Syzygium aromaticum (L.) Merrill & L.M. Perry essential oil, commonly known as clove essential oil, has gained attention because of its high eugenol content, a compound renowned for its potent insecticidal properties (Batiha et al. 2020). Eugenol and its derivatives have shown significant insecticidal effects against various insects, including important vectors such as *Aedes aegypti* (L.), *Sitophilus zeamais* (Motschulsky), and *Reticulitermes speratus* (Kolbe) (Barbosa et al. 2012, Fernandes et al. 2020, Prates et al. 2019). *Syzygium aromaticum* essential oil offers several advantages over synthetic insecticides, such as its mild effects on nontarget organisms and its low persistence in the environment, making it an environmentally friendly option for pest management (Isman et al. 2011). Derived from the buds of *S. aromaticum*, its essential oil has a rich history of traditional medicinal use and is recognized for its diverse pharmacological effects (Pérez et al. 2010).

Stored grains are vulnerable to infestations by *O. surinamensis*, which can lead to significant economic losses and health risks. This beetle species not only reduces the quality and quantity of stored grains but also contaminates them with foreign matter, rendering them unfit for consumption (Perera and Karunaratne 2015). In response to the challenges posed by synthetic fumigants and the development of resistance in pests, there is a growing interest in natural alternatives for pest management. This study aims to evaluate the chemical composition and effectiveness of *S. aromaticum* essential oil against adult *O. surinamensis*. By investigating its insecticidal properties and potential as a bioinsecticide, valuable insights can be gained into the practical application of *S. aromaticum* essential oil in sustainable pest management practices for stored grains.

Materials and Methods

Insect rearing. The study commenced with the collection of adult *O. surinamensis* from rice grains infested in storage facilities. These insects were subsequently reared in 10-l plastic buckets, each containing 2 kg of rice grains (*Oryza sativa* L.). Fifty adult *O. surinamensis* were introduced into each plastic bucket. The buckets were maintained under laboratory conditions at 30°C ± 5°C and relative humidity of 70% ± 5%. For the experiments, adults that were 2 weeks old

were selected. All experimental procedures were carried out under the same environmental conditions as those maintained for the insect cultures.

Essential oil extraction. The process began with the crushing of dried flower buds of *S. aromaticum* into small pieces through coarse grinding before they underwent the water distillation process. Next, 100 g of *S. aromaticum* was placed into a 2-l round flask and mixed with 1 l of distilled water. This mixture then underwent the distillation process using essential oil extraction equipment at a temperature range of 100–150°C for 6 h. After the distillation process, the essential oil separated and floated on the water. This fraction was then purified through centrifugation at 8,000 revolutions per min (rpm) for 10 min to remove any remaining water residues. The resulting essential oil of *S. aromaticum* was stored in an amber glass bottle at 4°C until used for analysis and bioassays.

Chemical composition analysis. The analysis of *S. aromaticum* essential oil followed the protocol described by Satongrod et al. (2021) using a gas chromatograph–mass spectrometer (GC-MS) (Clarus 680, PerkinElmer, USA). The column used was an Rtx-5MS capillary type, measuring 30 m in length and 0.32 mm in diameter, with a thickness of 1 μm. The essential oil was introduced in split mode (split ratio, 1:100 v/v) at a concentration of 100,000 parts per million, with a volume of 1 μl and helium gas as the carrier gas at a flow rate of 1.0 ml/min. The injector temperature was set at 280°C. Initially, the column temperature was maintained at 45°C for 5 min, followed by an increase in temperature at a rate of 10°C/min until it reached 200°C, where it was held for 5 min. The analysis utilized electron impact mode MS conditions with 70 eV, utilizing a quadrupole mass analyzer. The detector temperature was set at 250°C. The substances were characterized by comparing spectra using the National Institute of Standards and Technology Mass Spectral Search Program and the ChemStation Wiley Spectral Library. Compounds were considered similar to known substances if their mass spectra exhibited a match of more than 80%. The chemical composition data were analyzed on the basis of the retention time readings and percent peak area.

Fumigation toxicity. The experiment was designed using a completely randomized design (CRD) with four replicates. The experiments were carried out under laboratory conditions at a temperature of 30°C ± 5°C and a relative humidity of 70% ± 5%. The testing was conducted in glass fumigant test tubes, each with a cap and a volume of 40 ml, following the method outlined by Wanna et al. (2021) using the vapor-phase test method. To prepare the *S. aromaticum* essential oil solution for testing, it was diluted with acetone solvent (100% acetone) to obtain six concentrations: 0, 10, 20, 30, 40, and 50 μl/l of air. Subsequently, 50 μl of the *S. aromaticum* essential oil was pipetted onto a 1 × 2-cm filter paper (Whatman no. 4). The filter paper was allowed to evaporate at room temperature for 2 min before being placed under the lid of a fumigation test tube. Twenty adults of *O. surinamensis* were released into each tightly sealed fumigation test tube. The number of dead adult *O. surinamensis* was recorded at 1 h and every 6 h continuously up to 72 h. Subsequently, data were recorded every 24 h continuously until 168 h had elapsed.

Repellent effect. The fumigation effectiveness of *S. aromaticum* essential oil against adult *O. surinamensis* was examined to assess its insect-repellent properties. The experiments were conducted using a set of fumigation test tubes (8 cm in

diameter, 17 cm in height) equipped with covers. The vapor-phase method with choice test was utilized for the investigation. The fumigation test tube set consisted of one main test tube and one alternative tube. Each tube had a hole drilled at the bottom to insert a small plastic tube (0.5 cm in diameter, 10 cm in length) serving as a connection between them. An opening was drilled in the middle of the connecting pipe, fitted with a valve for releasing adult *O. surinamensis* into the test environment. To prepare the solutions of *O. surinamensis* essential oil for testing, the essential oils were diluted with 100% acetone solvent to achieve five concentrations: 0, 0.25, 0.5, 0.75, and 1 μl/l of air. A volume of 100 μl of each test essential oil solution was then dropped onto a sheet of filter paper (Whatman no. 4) 1.5 cm in width and 5 cm in length. The filter paper was allowed to evaporate at room temperature for 2 min before being placed into a small glass bottle (2.5 cm in diameter, 5 cm in height) suspended from the center of the tube cap. The plastic cap of the test tube was tightly closed. Similarly, an alternative tube was prepared following the same method, but with a piece of filter paper containing 100 μl of acetone only added to it. Subsequently, 20 adults of *O. surinamensis* were released into the middle opening of the connecting pipe between the test tubes. The opening was then completely sealed with a cover tube to prevent *O. surinamensis* from escaping outside the fumigation test tube set. The number of adult *O. surinamensis* found on both the test tube and alternative tube were recorded at intervals of 1, 3, 6, 12, and 24 h. Data collection was conducted every 24 h continuously for a total duration of 168 h.

Statistical analyses. The mortality of adult *O. surinamensis* was calculated on the basis of these recorded data by the following equation: % mortality $= (N\text{d}/N\text{t}) \times 100$ where *N*d is the number of dead adult *O. surinamensis* and *N*t is the total number of adult *O. surinamensis* used in the bioassay. If the mortality of adult *O. surinamensis* in the control treatment fell within the range of 5–20%, the mortality in each treatment needed to be adjusted using Abbott's formula (Abbott 1925). The fumigant toxicity, represented by the median lethal concentrations (LC_{50}) of *S. aromaticum* essential oil against adult *O. surinamensis*, was assessed using probit analysis. The data were expressed as repellent activity by using the following equation $PR = ([N\text{c} - N\text{t}]/[N\text{c} + N\text{t}]) \times 100$ where PR is percent repellency, *N*c is the number of adult *O. surinamensis* on the side of the alternative tube, and *N*t is the number of adult *O. surinamensis* on the side of the test tube. Statistical analysis of the data was performed using an *F*-test through one-way analysis of variance according to the CRD experimental design. The means were then compared using the least significant difference test ($P < 0.05$) by using Statistix, version 9.0 (Analytical Software, Tallahassee, Florida, USA).

Results

Chemical composition of *S. aromaticum* essential oil. *Syzygium aromaticum* essential oil consisted of a total of nine chemical components (99.78%), with the major constituent being eugenol (90.15%), followed by acetyleugenol (7.35%), β-caryophyllene (1.57%), caryophyllene oxide (0.26%), α-caryophyllene (0.25%), *p*-allylphenol (0.15%), methyl salicylate (0.03%), 4-hydroxy-4-methyl-2-pentanone (0.01%), and α-copaene (0.01%). The components and their percentages are listed in Table 1.

Table 1. Chemical composition of essential oil obtained from flower buds of *Syzygium aromaticum*.

No.	Compound	Retention Time (min)	% Peak Area
1	4-Hydroxy-4-methyl-2-pentanone	3.217	0.01
2	Methyl salicylate	13.778	0.03
3	*p*-Allylphenol	16.561	0.15
4	Eugenol	22.251	90.15
5	β-Caryophyllene	23.494	1.57
6	α-Caryophyllene	24.760	0.25
7	α-Copaene	25.393	0.01
8	Acetyleugenol	27.660	7.35
9	Caryophyllene oxide	29.644	0.26
	Total		99.78

Fumigation activity. The *S. aromaticum* essential oil exhibited strong toxicity against adult *O. surinamensis*. The LC_{50} for the insect population was observed at 24 h of exposure, with a value of 9.70 μl/l of air. As the concentration of essential oil increased, the mortality rate of adult *O. surinamensis* also increased. Moreover, with longer exposure times to *S. aromaticum* essential oil, the mortality rate rose, and the LC_{50} decreased to 6.58 and 4.37 μl/l of air at 48 and 72 h, respectively (Table 2).

Additionally, when adult *O. surinamensis* were exposed to various concentrations of *S. aromaticum* essential oil, their average mortality (%) varied at 10, 20, 30, 40, and 50 μl/l of air. The efficacy of *S. aromaticum* essential oil against adult *O. surinamensis* showed significant differences between 24 and 96 h. Notably, at 24 and 48 h, the highest efficiency in killing adult *O. surinamensis* was observed with *S. aromaticum* essential oil at 40 μl/l of air, resulting in a mortality rate of

Table 2. Fumigant toxicity (LC_{50})[a] of *Syzygium aromaticum* essential oil against *Oryzaephilus surinamensis*.

Time (h)	*n*	LC_{50} (μl/l of Air)	95% CI	Regression Equation	r^2
24	480	9.70	8.07–10.40	$y = 5.4464x - 2.8571$	0.97
48	480	6.58	5.80–7.65	$y = 7.3929x + 1.369$	0.99
72	480	4.37	3.25–5.31	$y = 9.2143x + 9.7619$	0.97

[a] LC_{50} (50% lethal concentration) is the concentration of *S. aromaticum* essential oil required to kill half of the members of *O. surinamensis* adults tested after a specified test duration period, expressed in μl/l of air; *n* is number of *O. surinamensis* adults tested; CI is confidence interval; r^2 is coefficient of determination.

>90%. This concentration showed no statistically significant difference compared with 50 μl/l of air (Table 3). Furthermore, at 72 and 96 h, *S. aromaticum* essential oil at 20 and 10 μl/l of air, respectively, achieved an eradication efficiency of adult *O. surinamensis* (97.5% and 98.75%) similar to that of 50 μl/l of air, with no significant differences observed.

Repellent effect. The repellent effect of *S. aromaticum* essential oil was investigated at four concentrations (0.25, 0.5, 0.75, and 1 μl/l of air) against adult *O. surinamensis*. The results showed a significant difference in the repellency of adult *O. surinamensis* in the alternative tubes (Table 4), with a range of 73.75% to 91.25% within 120 h after the bioassay. Higher concentrations demonstrated increased repellency against adult *O. surinamensis*. The results of these experiments clearly demonstrate that the highest repellent activity was observed at a concentration of 1 μl/l of air.

Discussion

This study confirms that eugenol is the main component of *S. aromaticum* essential oil, which is consistent with the findings of Rashmi (2022) regarding eugenol, eugenol acetate, β-caryophyllene, and α-humulene. The essential oil was obtained through steam distillation, with GC and GC-MS analysis revealing approximately 87% eugenol, 8.01% eugenyl acetate, and 3.56% β-caryophyllene as the main components. Similar reports by Affonso et al. (2012), Batiha et al. (2020), Boughendjioua (2018), Fuentes et al. (2020), Haro-González et al. (2021), and Jumbo et al. (2018) confirm eugenol as the major compound, ranging from 66.9% to 90.41%. Studies also indicate the presence of phenolic compounds with various biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties. The composition of *S. aromaticum* oil can vary because of genetic, climatic, cultivation factors (Gioffrè et al. 2020, Mohamed et al. 2021, Rashmi 2022), and storage conditions (duration, temperature, and humidity) (Cavar Zeljković et al. 2021, Hu et al. 2022). These findings underscore the potential of *S. aromaticum* essential oil as a bioactive compound, urging further investigation into its applications and efficacy.

The study on *S. aromaticum* essential oil reveals its remarkable toxicity against adult *O. surinamensis*, evident from the decreasing LC_{50} values with increased exposure time. This is consistent with the findings of Jairoce et al. (2016), who reported high mortality rates and corresponding LC_{50} values in *Si. zeamais* and *Acanthoscelides obtectus* (Say). Similarly, Plata-Rueda et al. (2018) highlighted the effectiveness of eugenol in controlling *Si. granarius*, resulting in significant reductions in insect respiratory and mobility rates. Additionally, Cardiet et al. (2012) observed potent insecticidal activity of *S. aromaticum* essential oil against *Si. oryzae*, with notable mortality rates at specific concentrations. The mode of action of *S. aromaticum* essential oil involves its impact on octopamine, γ-aminobutyric acid receptors, and transient receptor potential channels, leading to increased mortality rates at higher concentrations. This disrupts essential cell and cytoplasmic membranes, inhibiting key enzymes such as acetylcholinesterase and resulting in uncoordinated movements in insects. Monoterpenes and eugenol found in the essential oil play crucial roles in its insecticidal and repellent

Table 3. Mortality of *Oryzaephilus surinamensis* after fumigation bioassay with *Syzygium aromaticum* essential oil.

	Mortality (%) ± SE of *O. surinamensis*				
Concentration (µl/l of Air)	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**
0	0.00 ± 0.00e*	0.00 ± 0.00e	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00
10	70.00 ± 4.08d	77.50 ± 2.89d	90.00 ± 4.08b	98.75 ± 2.50a	100.00 ± 0.00
20	78.75 ± 2.50c	87.50 ± 2.89c	97.50 ± 5.00a	100.00 ± 0.00a	100.00 ± 0.00
30	86.25 ± 2.50b	93.75 ± 2.50b	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00
40	90.00 ± 4.08ab	98.75 ± 2.50a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00
50	93.75 ± 2.50a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00
F-test	**	**	**	**	N/A
Coefficient of variation (%)	4.22	2.89	3.24	1.23	—
Least significant difference	4.38	3.27	3.91	1.52	—

* Means within the same column followed by the same letter are not significantly different (least significant difference: $P > 0.05$).
** Significant difference at $P < 0.01$.

Table 4. Repellent activity of *Syzygium aromaticum* essential oil against *Oryzaephilus surinamensis* after fumigation bioassay.

Concentration (μl/l of air)	Repellent Activity (%) ± SE of *Oryzaephilus surinamensis*				
	24 h	48 h	72 h	96 h	120 h
0.25	73.75 ± 2.50c*	76.25 ± 2.50c	77.50 ± 2.89c	78.75 ± 2.50c	80.00 ± 0.00c
0.5	78.75 ± 2.50b	80.00 ± 0.00b	82.50 ± 2.89b	85.00 ± 0.00b	85.00 ± 0.00b
0.75	83.75 ± 2.50a	83.75 ± 2.50a	85.00 ± 0.00ab	87.50 ± 2.89b	88.75 ± 2.50a
1	86.25 ± 2.50a	86.25 ± 2.50a	87.50 ± 2.89a	91.25 ± 2.50a	91.25 ± 2.50a
F-test	**	**	**	**	**
CV (%)	3.10	2.65	3.01	2.67	2.05
Least significant difference	3.85	3.34	3.85	3.52	2.72

* Means within the same column followed by the same letter are not significantly different (least significant difference: $P > 0.05$).
** Significant difference at $P < 0.01$.

properties, also regulating insect respiration (Dahake and Kanase 2017, Ghosh and Narasimhan 2014, Narayanan and Muraleedharan 2017, Priestley et al. 2003). Recent studies by Mussalama et al. (2023) highlighted the potential of clove and thyme essential oils against *Ac. obtectus*, showing significant mortality rates and suggesting their efficacy in storehouses. Additionally, Ainane et al. (2019) investigated the repellent properties of eugenol, with promising results in toxicity against *Tribolium confusum* Duval. Mishra et al. (2021) further emphasized the insecticidal activity of *S. aromaticum* essential oil against *Si. oryzae*, supporting its potential as a pest management solution. Further discussions from recent studies include those by Ahmed et al. (2022), who investigated the neurotoxic effects of *S. aromaticum* essential oil on insect pests, highlighting its disruption of key neurotransmitter systems. Patel et al. (2023) provided insights into the mechanism of action of eugenol, emphasizing its role in inhibiting vital enzymes and disrupting insect nervous systems. Additionally, Lee et al. (2021) discussed the potential of *S. aromaticum* essential oil as a biopesticide because of its selective toxicity against pests and minimal impact on beneficial insects. Furthermore, Kumar et al. (2024) explored the repellent properties of *S. aromaticum* essential oil, showcasing its effectiveness in deterring stored-product pests from infesting grains.

These findings collectively underscore the promising potential of *S. aromaticum* essential oil in IPM strategies. Its multifaceted mode of action, coupled with its repellent properties and minimal impact on nontarget organisms, position it as a valuable tool in sustainable pest control practices. Further research into its specific mechanisms and optimal application methods could enhance its efficacy and promote its widespread adoption in agricultural settings.

Acknowledgments

This research project was financially supported by Mahasarakham University, Maha Sarakham, Thailand. The authors acknowledge the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Maha Sarakham, Thailand for laboratory support. Laboratory assistance was provided by Jiraporn Krasaetep.

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